# Cerebral formation of free radicals during hypoxia does not cause structural damage and is associated with a reduction in mitochondrial PO2; evidence of O2-sensing in humans?

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Abstract: Cellular hypoxia triggers a homeostatic increase in mitochondrial free radical signaling. In this study, blood was obtained from the radial artery and jugular venous bulb in 10 men during normoxia and 9 hours hypoxia (12.9% O(2)). Mitochondrial oxygen tension (p(O(2))(mit)) was derived from cerebral blood flow and blood gases. The ascorbate radical (A(•-)) was detected by electron paramagnetic resonance spectroscopy and neuron-specific enolase (NSE), a biomarker of neuronal injury, by enzyme-linked immunosorbent assay. Hypoxia increased the cerebral output of A(•-) in proportion to the reduction in p(O(2))(mit), but did not affect NSE exchange. These findings suggest that neuro-oxidative stress may constitute an adaptive response.

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# Cerebral formation of free radicals during hypoxia does not cause structural damage and is associated with a reduction in mitochondrial $PO_2$; evidence of $O_2$-sensing in humans?

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ABSTRACT

Cellular hypoxia triggers a homeostatic increase in mitochondrial free radical signalling. In this study, blood was obtained from the radial artery and jugular venous bulb in ten males during normoxia and 9h hypoxia (12.9%$O_2$). Mitochondrial oxygen tension ($\bar{P}_{O_2}^{mit}$) was derived from cerebral blood flow and blood gases. The ascorbate radical ($A^{\bullet-}$) was detected by electron paramagnetic resonance spectroscopy and neuron-specific enolase (NSE), a biomarker of neuronal injury, by ELISA. Hypoxia increased the cerebral output of $A^{\bullet-}$ in proportion to the reduction in $\bar{P}_{O_2}^{mit}$ but did not affect NSE exchange. These findings suggest that neuro-oxidative stress may constitute an adaptive response.

## INTRODUCTION

Although the human brain represents only 2% body weight it accounts for a disproportionate 25% of basal oxygen ($O_2$) consumption to support the demands of neuronal activity. This commitment to an obligatory high rate of $O_2$ consumption combined with limited glycolytic capacity renders the brain exquisitely sensitive to $O_2$ deprivation (hypoxia) with a correspondingly high vulnerability for failure (Bailey *et al* 2009a). As a consequence, elaborate cellular $O_2$-sensing mechanisms have evolved to preserve $O_2$ homeostasis and ultimately prevent brain damage (Sharp and Bernaudin 2004).

However, despite considerable progress in our understanding of the cerebral pathways activated during hypoxia, a unified mechanism that allows the brain to sense and ultimately respond to $O_2$ remains elusive. Emerging evidence suggests that the mitochondrion may function as the primary $O_2$ "sensor" given its capacity to increase free radical and associated reactive oxygen-nitrogen species (ROS-RNS) formation during hypoxia (Chandel *et al* 1998). Though thermodynamically capable of causing structural damage when in excess, these oxidant signals can act as second messengers (Wright *et al* 2004) that activate $O_2$ salvage genes through stabilization of the transcription factor hypoxia-inducible factor (HIF)-1$\alpha$ (Guzy and Schumacker 2006) in physiologically controlled though as of yet undefined amounts.

In support, human studies have documented an increased systemic formation of free radicals during hypoxia in proportion to arterial desaturation (Bailey *et al* 2009b). However, whether any potential relationship exists between cerebral mitochondrial $PO_2$ and the focal (cerebral) formation of free radicals remains to be examined. This is clinically relevant given the cerebral complications encountered in diseases defined

by hypoxemia, notably obstructive sleep apnea and chronic obstructive pulmonary disease (Sharp and Bernaudin 2004).

To investigate this, we combined electron paramagnetic resonance (EPR) spectroscopy for direct detection of the abscorbate radical ($A^{\bullet-}$) with calculation of cerebral mitochondrial $PO_2$ ($\bar{P}_{O_2}^{mit}$) in a group of healthy volunteers exposed to hypoxia. We hypothesized that compared to normoxia, hypoxia would promote the cerebral formation and net output of $A^{\bullet-}$ as indicated by a negative arterio-jugular venous concentration difference ($a\text{-}jv_D$). Since we considered this to reflect an adaptive response that preserves $O_2$ homeostasis, we further hypothesized that $A^{\bullet-}$ output would correlate against the reduction in $\bar{P}_{O_2}^{mit}$ in the absence of neurovascular tissue damage as indicated by a lack of exchange in the neuronal-parenchymal injury biomarker, neuron-specific enolase (NSE).

## METHODS

### *Subjects and design*

Ten males aged 27 ± 4 years old provided written informed consent following approval by the Scientific Ethics Committee of Copenhagen and Frederiksberg Municipalities (Denmark). Following local anesthesia (2% lidocaine), subjects received a retrograde catheter in the right internal jugular vein via the Seldinger technique under ultrasound guidance with the tip advanced to the bulb of the vein. A radial arterial catheter was placed in the non-dominant arm. Baseline samples were collected in normoxia (21%$O_2$) and after 9h passive exposure to hypoxia (12.9%$O_2$).

### *Cerebral oxygenation*

Hemoglobin (Hb), hematocrit (Hct), partial pressure of oxygen and carbon dioxide ($PO_2$/$PCO_2$), oxyhemoglobin saturation ($SO_2$), pH, P50, temperature and (whole-blood) glucose were determined using a blood gas analyzer (ABL 715 Radiometer Medical, Denmark). Global cerebral blood flow (CBF) was measured via the Kety-Schmidt technique in the de-saturation mode using nitrous oxide ($N_2O$) as the tracer (Taudorf *et al* 2009). Briefly, subjects inhaled 5% $N_2O$ and $O_2$ in $N_2$ (corresponding to both normoxic and hypoxic inspirates) for 30 minutes. From two minutes before the cessation of $N_2O$ until 15 minutes thereafter, 12 sets of paired synchronized samples were drawn into gas-tight syringes from the arterial and venous catheters. Samples equilibrated against air in a water bath at 37ºC for 60 minutes and $N_2O$ concentration measured via photoacoustic spectroscopy (Innova Photoacoustic Field Gas Monitor 1412, Brüel & Kjaer, Denmark). Unlike our previous study (Taudorf *et al* 2009), all tubing was pre-saturated with a syringe filled with pure $N_2O$, $O_2$ and $N_2$

corresponding to the inspired air mixtures prior to running experimental samples. Global CBF was subsequently derived by the Kety-Schmidt equation given as:

$$CBF = 100 \times \lambda \times \frac{c_{jv}\ (equilibrium)}{\int_{t=0}^{t=\infty} (c_{jv}(t)\ x\ dt)\ \int_{t=0}^{t=\infty} (c_a(t)\ x\ dt)}$$

$$= 100 \times \lambda \times \frac{height\ at\ equilibrium}{srea\ between\ curves}$$

where $c_{jv}$ ($t$) and $c_a$ ($t$) are the jugular venous and arterial concentration of the tracer at time ($t$) in min and $\lambda$ represents the brain-blood partition coefficient (ml/g) calculated from data reported previously (Kety *et al* 1948):

$$\lambda = 1.167 - [0.0026 \times Hct\ (\%)]$$

Cerebral plasma flow (CPF) was calculated as CBF x (1-Hct). Cerebral $O_2$ delivery was calculated as:

$$CBF \times c_aO_2$$

where $c_aO_2$ refers to arterial oxygen content (mmol):

$$c_aO_2 = 0.60 \times S_aO_2 \times Hb + 0.0013 \times P_aO_2$$

The oxygen extraction fraction ($EO_2$) was determined by:

$$EO_2 = \frac{c_aO_2 - c_vO_2}{c_aO_2} \; x \; 100 \; (\%)$$

The global cerebral metabolic rate of oxygen ($CMRO_2$) was calculated as:

$$CMRO_2 = CBF \times a\text{-}jv_DO_2$$

where $a\text{-}jv_DO_2$ refers to the arterio-jugular venous $O_2$ content difference.

The oxygen-glucose index (OGI) was calculated as:

$$OGI = \frac{c_aO_2 - c_vO_2}{c_a\text{glucose} - c_v\text{glucose}}$$

Using established formalism (Gjedde 2005; Gjedde *et al* 2005; Rasmussen *et al* 2007), the mean capillary oxygen saturation ($\bar{S}_{O2}^{cap}$) was calculated as:

$$\bar{S}_{O_2}^{cap} = SaO_2 \left(1 - \frac{c_aO_2 - c_vO_2}{2c_aO_2}\right)$$

assuming that $O_2$ extraction rises linearly with distance as blood traverses the capillary network. Solution of the Hill equation permitted calculation of the (mean) capillary $PO_2$ ($\bar{P}_{O_2}^{cap}$):

$$\bar{P}_{O_2}^{cap} = P_{50a}^{Hb} \sqrt[h_a]{\frac{\bar{S}_{O_2}^{cap}}{1-\bar{S}_{O_2}^{cap}}}$$

where $P_{50a}^{Hb}$ is the $PO_2$ when Hb is half-saturated (29 mmHg) and $h_a$ is Hill's coefficient (2.84 for arterial blood). Given the absence of capillary recruitment in the brain, $O_2$ diffusibility (L) was calculated during hypoxia (in which the mitochondrial oxygen tension could be assumed to be negligible) and given as:

$$L = \frac{CMRO_2}{\bar{P}_{O_2}^{cap}}$$

based on group mean values and assumed to be constant at 7.8 $\mu$mol $100g^{-1}min^{-1}mmHg^{-1}$ for any given CBF. The mean mitochondrial $PO_2$ ($\bar{P}_{O_2}^{mit}$) was subsequently given by:

$$\bar{P}_{O_2}^{mit} = \bar{P}_{O_2}^{cap} - \frac{CMRO_2}{L}$$

The assumptions and limitations associated with the formalism underlying the nonlinear flow-metabolism coupling model have been discussed in detail elsewhere (Gjedde 2005).

*Ascorbate (antioxidant)*

Exactly 100 $\mu$L of plasma was stabilized and deproteinated by adding 900 $\mu$L of 5% metaphosphoric acid (Sigma Chemical, Dorset, UK). Ascorbate was assayed by fluorimetry based on the condensation of dehydroascorbic acid with 1,2-phenylenediamine (Bailey *et al* 2009d). The intra- and inter-assay CV were both <5 %.

*Ascorbate (free radical)*

Exactly 1mL of plasma was injected into a high-sensitivity multiple-bore sample cell (AquaX, Bruker Instruments Inc, Billerica, USA) housed within a $TM_{110}$ cavity and analyzed by X-band EPR spectroscopy operating at 100 kHz modulation frequency, 0.65 Gauss (G) modulation amplitude, 10 mW microwave power, 2 x $10^5$ receiver gain and 41 ms time constant for 3 incremental scans (Bailey *et al* 2009b). Spectra were filtered identically using WINEPR (Version 2.11) and the double integral of each doublet was calculated using Origin software (Version 5.0). The intra- and inter-assay CV were both <5 %.

*Neuron specific enolase (NSE)*

NSE was incorporated as a molecular biomarker of neuronal-parenchymal injury using a commercially available ELISA (CanAg, Ref 420-10) with a lower detection limit of <1$\mu$g/L. The intra and inter-assay CV were both <5 %.

*Cerebral net exchange*

This was calculated as:

$$\text{CPF} \times a\text{-}jv_D$$

By convention, a negative value reflects net cerebral output or gain whereas a positive value reflects consumption or loss.

*Cephalalgia*

Symptoms of acute mountain sickness (AMS) were assessed using the Lake Louise (LL) (Roach *et al* 1993) and Environmental Symptoms Questionnaire Cerebral (ESQ-C) (Sampson *et al* 1983) scoring systems. A clinically-validated visual analog scale (VAS) was employed to measure headache (Iversen *et al* 1989). Clinical AMS was diagnozed if a subject presented with a combined total LL score (self assessment + clinical score) of $\geq 5$ points and ESQ-C score $\geq 0.7$ points (Bailey *et al* 2009b).

*Statistical Analysis*

Shapiro-Wilk *W* tests confirmed distribution normality. Cerebral oxygenation and exchange data were analyzed using a combination of paired samples *t*-tests and 2-way repeated measures ANOVA. Relationships were examined using a Pearson Product Moment Correlation. Significance was established at $P < 0.05$ and data are presented as mean ± SD.

RESULTS

*Cerebral oxygenation*

Hypoxia was associated with a reduction ($P < 0.05$ *vs.* normoxia) in $PaO_2$ (107 ± 6 to 46 ± 3 mmHg), $PaCO_2$ (43 ± 2 to 35 ± 2 mmHg), $SaO_2$ (99 ± 1 to 83 ± 3 %), and corresponding cerebral $O_2$ delivery (Table 1). As a consequence, $\bar{S}_{O_2}^{cap}$, $\bar{P}_{O_2}^{cap}$ and $\bar{P}_{O_2}^{mit}$ were also shown to decrease. An increase in $EO_2$ and (tendency towards) CBF counteracted hypoxemia thus preserving $CMRO_2$. By contrast, the OGI was not altered during hypoxia (6.11 ± 0.79 *vs*. 5.86 ± 0.95 in normoxia, $P > 0.05$).

*Free radicals*

Hypoxia did not affect the $a\text{-}jv_D$ or subsequent exchange of ascorbate (hypoxia: 0.052 ± 0.250 *vs*. normoxia: 0.120 ± 0.249 mM $100g^{-1}$ $min^{-1}$, $P > 0.05$). In contrast, hypoxia decreased the $a\text{-}jv_D$ of $A^{\bullet-}$ (-522 ± 471 *vs*. -148 ± 256 AU $100g^{-1}$, $P < 0.05$) due primarily to an increase in jugular venous outflow ($P < 0.05$). Typical examples of $A^{\bullet-}$ spectra ($a^{H4}$ ≈1.76 G, $\Delta H_{pp}$ = ≈0.6G and $g$ = 2.0052) observed with corresponding signal intensities are illustrated in Figure 1A. This translated into an increased cerebral output in hypoxia (-29283 ± 25852 *vs*. -7131 ± 12260 AU $100g^{-1}min^{-1}$, $P < 0.05$) which correlated directly against the reduction in $\bar{P}_{O_2}^{mit}$ (Figure 1B).

*Neurovascular integrity*

There was no evidence of neuronal-parenchymal injury since hypoxia affected neither the $a\text{-}jv_D$ nor the exchange of NSE (34.6 ± 40.2 *vs*. normoxia: 30.2 ± 36.3 $\mu$g $100g^{-1}min^{-1}$, $P > 0.05$).

*Cephalalgia*

Hypoxia increased AMS (LL: 3 ± 2 *vs*. normoxia: 0 ± 0 points, $P < 0.05$; ESQ-C: 0.803 ± 0.674 *vs*. normoxia: 0.000 ± 0.000 AU, $P < 0.05$) and headache (VAS: 29 ± 23 *vs*. normoxia: 0 ± 0 mm, $P < 0.05$) scores which correlated modestly against the increased cerebral output of $A^{\bullet-}$ (LL: $r = -0.61$, $P = 0.06$; ESQ-C: $r = -0.56$, $P = 0.09$; VAS: $r = -0.65$, $P = 0.04$). Furthermore, the one subject diagnozed with clinical AMS exhibited the greatest reduction in $\bar{P}_{O_2}^{mit}$ (-30.9 mmHg) and increase in the $a\text{-}jv_D$ (-1334 AU $100g^{-1}$) and subsequent cerebral output of $A^{\bullet-}$ (-75424 AU $100g^{-1}min^{-1}$) during hypoxia.

## DISCUSSION

The major finding in the present study was that the increased cerebral output of free radicals in the form of $A^{\bullet-}$ observed during hypoxia did not compromize neuronal-parenchymal integrity and correlated directly against the reduction in $\bar{P}_{O_2}^{mit}$. While this does not disassociate cause from effect, it points towards an adaptive mechanism by which mitochondria potentially "sense" changes in cerebral $O_2$ tension through free radical formation with the capacity to activate signaling pathways that serve to defend $O_2$ homeostasis in the face of a reduced $PO_2$.

These findings extend previous EPR spin-trapping reports of an increased cerebrospinal fluid (CSF) concentration (Bailey *et al* 2006) and net cerebral output of blood-borne alkoxyl-alkyl radicals during hypoxia (Bailey *et al* 2009d). In the current study, we took advantage of $A^{\bullet-}$ as a biomarker to determine the brain's "global" rate of free radical flux *in-vivo* rather than focus on select species trapped *ex-vivo* whose individual contributions to the neuro-oxidative cascade remain, as a consequence, uncertain. Given that the one-electron reduction potential associated with the $A^{\bullet-}$ /ascorbate monanion ($AH^-$) couple is so low ($E^{O'}$ = +282mV at pH 7.0), any radical generated across the cerebral circulation will react endogenously with this terminal small-molecule antioxidant to form the distinctive doublet $A^{\bullet-}$ that is readily detectable by EPR [($R^{\bullet} + AH^- \rightarrow A^{\bullet-} + R\text{-}H$, (Buettner 1993)]. The fact that hypoxia did not affect ascorbate exchange further emphasizes that the increased output of $A^{\bullet-}$, detectable even within the time constraints of a single arterio-venous transit, likely reflects *de-novo* radical formation (and not simply increased ascorbate auto-oxidation) by any number of oxidizing species whose identities remain to be established.

Though beyond the scope of the present study, the source, mechanisms and functional significance of free radical formation in hypoxia remains to be established. Given its

modest antioxidant defenses, abundance of catalytic transition metals, auto-oxidizable neurotransmitters and neuronal membrane lipids rich in polyunsaturated fatty acid side-chains exposed to a disproportionately high $O_2$ flux, the human brain has traditionally been considered especially vulnerable to molecular attack (Bailey *et al* 2009a). To examine this, we measured exchange of the dimeric enzyme NSE, the neuronal form of the glycolytic enzyme enolase (2-phospho-*D*-glycerate hydrolase) which is located almost exclusively within neuronal cell bodies, axons and neuroendocrine cells (Schmechel *et al* 1978). Its appearance across the cerebral circulation would therefore reflect parenchymal tissue injury in direct proportion to the extent of damage (Schoerkhuber *et al* 1999). Thus, the lack of exchange observed in the current study combined with prior reports that have consistently failed to document any increase in the systemic (Bailey *et al* 2009b), CSF (Bailey *et al* 2006) and trans-cerebral (Bailey *et al* 2009d) concentration of the astrocytic protein S100*β*, a peripheral biomarker of blood-brain barrier damage, confirms that cerebral free radical formation proceeds in hypoxia without compromizing neurovascular integrity.

As anticipated, hypoxia was associated with AMS and headache with modest correlations observed between symptom scores and the increased cerebral output of $A^{\bullet-}$. Similar correlations have been observed between the rise in (cephalic) venous $A^{\bullet-}$ and cephalalgia which could be explained, at least in part (≈30% of the variance) by the reduction in arterial oxyhemoglobin saturation (Bailey *et al* 2009b). Thus, if AMS is indeed associated with more marked arterial hypoxemia as previously suggested (Ge *et al* 1997), symptoms may simply prove the consequence of more marked $\bar{P}_{O_2}^{mit}$-induced cerebral ROS-RNS formation which although controversial (Bailey *et al* 2009c), have the capacity to sensitize perivascular sensory nerves and activate trigeminovascular nocioceptors (Sanchez del Rio and Moskowitz 1999). Follow-up

exchange studies employing "brain-specific" targeted antioxidants are warranted to confirm if neuro-oxidative-nitrosative-stress is indeed a causal or merely consequential event and its corresponding impact on cerebral oxygenation.

These findings force a re-appraisal of free radicals as simply damaging or toxic "accidents of chemistry" and argue that their formation by the human brain may represent an adaptive response to hypoxia. It is tempting to speculate that the increases in CBF and $EO_2$ observed in the current study were subject to redox-regulation and served to maintain global cerebral oxygenation in the face of severe hypoxemic-hypocapnia. This concept is consistent with earlier work implicating (mitochondrial) ROS-RNS as signalling molecules that coordinate the intrinsic mitochondrial cell death pathway in response to a diverse range of cellular stressors (Wright *et al* 2004). More recently, *in-vitro* studies which have identified the electron transport chain as the primary $O_2$ sensor, with Complex III-derived ROS released to the inter-membrane space and cytosol as signals capable of triggering transcriptional and post-translational responses to hypoxia, notably stabilization of HIF-1$\alpha$, that ultimately preserve cellular $O_2$ supply (Waypa *et al* 2010). In support, antioxidant prophylaxis was recently associated with a reduction in brachial artery vasodilation in exercising humans (Richardson *et al* 2007), further establishing the functional importance of free radical formation in controlled, though as of yet undefined amounts.

It is important to recognize that our findings reflect "global" as opposed to "regional" cerebral responses to hypoxia and the techniques employed lack the temporal resolution required to assess dynamic change. The regional variation in the CBF response to acute (albeit isocapnic) hypoxia (8 mins at 10% $O_2$) was confirmed by $H_2{}^{15}O$ positron emission tomography (Binks *et al* 2008). The authors identified that

phylogenetically-older structures of the brain (nucleus accumbens, putamen, pallidum, caudate and thalamus) exhibited greater increases in CBF during hypoxia compared to evolutionary-younger (cortical) regions which they speculated may represent an adaptive response that serves to protect regions responsible for (the more) essential homeostatic functions. Thus, like the skeletal and pulmonary circulatory response to hypoxia, cerebral tissue is equally defined by marked diffusion-perfusion heterogeneity and as a consequence, one must therefore assume that the $\bar{P}_{O_2}^{mit}$ response to hypoxia follows a similar pattern in that regional variations likely predominate.

The values obtained for CBF and $CMRO_2$ (and thus by consequence the derivation of L) were noticeably higher than those reported in previous studies using the same technique, albeit with $^{133}Xe$ as the tracer (Madsen *et al* 1993; Moller *et al* 2002). The reasons for this discrepancy are not clear though we remain confident that they were not caused by the contamination of samples with blood from extra-cerebral regions or as a consequence of inadequate desaturation.

Extracranial contamination would lead to a higher CBF but would probably not affect the $CMRO_2$ or even lower the measured values given the low metabolic rate and oxygen extraction of extra-cranial tissues. This is further supported by the observed OGIs which would have been expected to be significantly greater than the observed values of ≈6 given that such tissue would not be expected to be fueled exclusively by glucose. Finally, in the absence of radiographic evidence, all subjects reported a "purl" during saline injection into the catheter which indirectly confirms correct line placement.

In terms of desaturation, since $N_2O$ is less soluble in biological tissues than $^{133}Xe$ (Kety *et al* 1948) the corresponding washout phase should theoretically be shorter and

thus the error of measurement less. Furthermore, the sampling period was expanded from the original 10 min as applied with $^{133}$Xe (Madsen *et al* 1993; Moller *et al* 2002) to 15 min to confirm complete washout. Finally, our choice of $N_2O$ as the tracer did not influence blood-borne nitric oxide (NO) metabolites thus arguing against NO-mediated vasodilatation as a potential contaminant (Taudorf *et al* 2009). Though the reasons underlying these atypically high CBF and $CMRO_2$ values remain unknown, the repeated-measures design ensured precision with our primary interest focused on the qualitative changes incurred by hypoxia.

In conclusion, the observation that hypoxia triggers a net cerebral output of free radicals in the absence of neurovascular tissue damage and in direct proportion to the reduction in $\bar{P}_{O_2}^{mit}$ provides the first tentative evidence for $O_2$-sensing by the human brain. It would be of interest to determine if antioxidant prophylaxis ablates the cerebral adaptive response to hypoxia in future studies.

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Table 1. Cerebral oxygenation

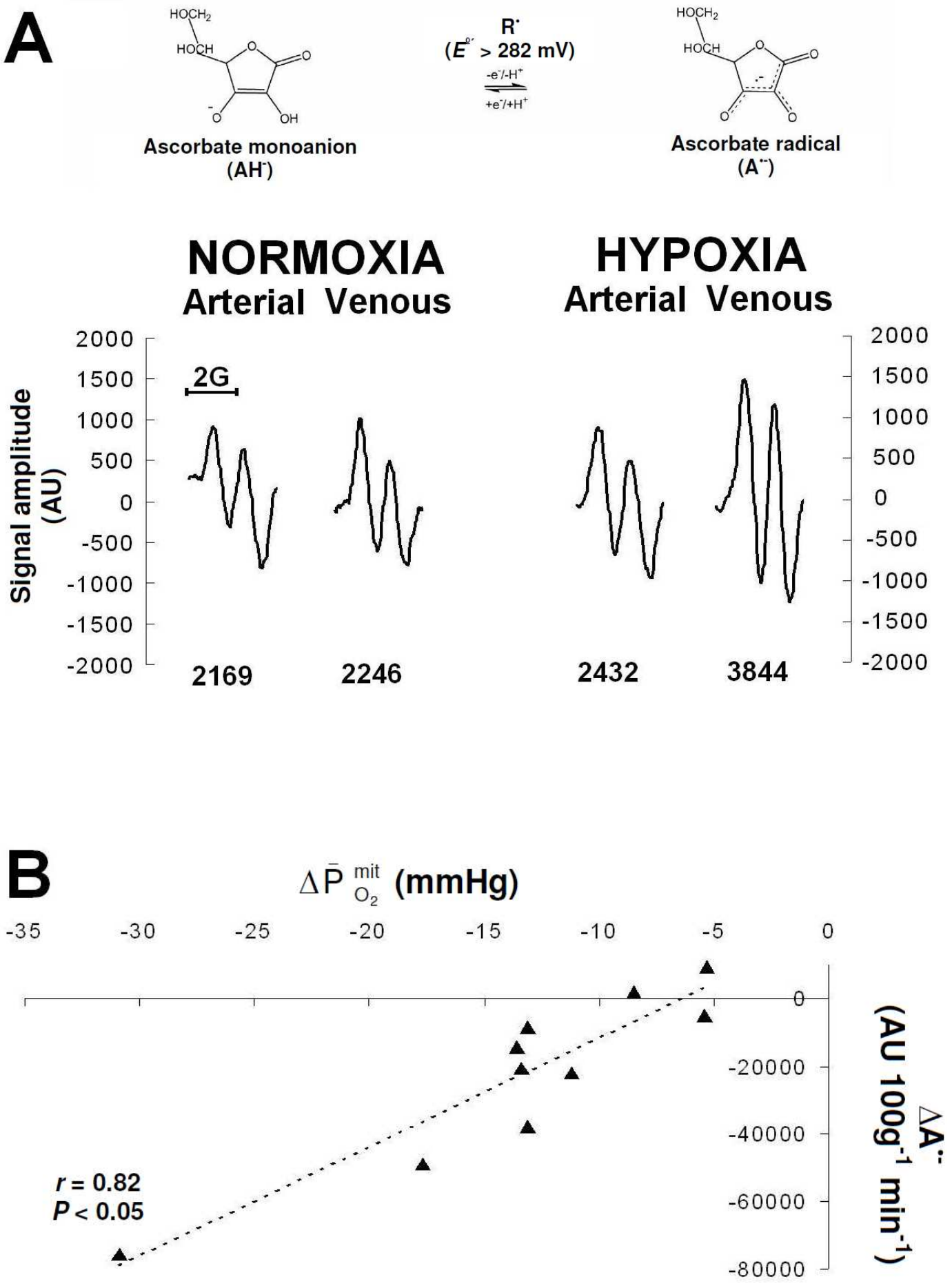
| | Normoxia | Hypoxia |
|---|---|---|
| CBF (ml $100g^{-1}min^{-1}$) | 85 ± 15 | 94 ± 17 |
| CPF (ml $100g^{-1}min^{-1}$) | 51 ± 9 | 57 ± 11 |
| $O_2$ delivery ($\mu$mol $100g^{-1}min^{-1}$) | 728 ± 145 | 666 ± 108 |
| $a\text{-}jv_DO_2$ (mmol) | 2.8 ± 0.3 | 2.7 ± 0.4 |
| $EO_2$ (%) | 33 ± 3 | 38 ± 6† |
| $CMRO_2$ ($\mu$mol $100g^{-1}min^{-1}$) | 243 ± 55 | 249 ± 34 |
| $\bar{S}_{O_2}^{cap}$ (%) | 82.7 ± 1.3 | 66.9 ± 3.6† |
| $\bar{P}_{O_2}^{cap}$ (mmHg) | 43.3 ± 2.0 | 33.3 ± 2.0† |
| $\bar{P}_{O_2}^{mit}$ (mmHg) | 14.6 ± 4.1 | 1.4 ± 5.5† |

Values are mean ± SD; CBF/CPF, cerebral blood/plasma flow; $a\text{-}jv_DO_2$, arterio-jugular venous $O_2$ content difference; $EO_2$, oxygen extraction fraction; $CMRO_2$, cerebral metabolic rate of oxygen; $\bar{S}_{O_2}^{cap}$, mean capillary $O_2$ saturation; $\bar{P}_{O_2}^{cap}$, mean capillary $PO_2$; $\bar{P}_{O_2}^{mit}$, mean mitochondrial $PO_2$; †different ($P < 0.05$).

Figure Legends

Figure 1A. Oxidation of the ascorbate monoanion ($AH^-$) by an initiating species ($R^\bullet$) with a one electron reduction potential ($E^{\circ\prime}$) greater than +282 mV yields the domesticated ascorbate radical ($A^{\bullet-}$). The unpaired electron is delocalized over a highly conjugated tri-carbonyl π-system rendering it resonance-stabilized facilitating direct detection by electron paramagnetic resonance (EPR) spectroscopy. Note the increase in the systemic and cerebral formation of $A^{\bullet-}$ during 9h passive exposure to hypoxia. Digits below each spectrum represent EPR signal intensities in arbitrary units (double integral which is directly proportional to the concentration of $A^{\bullet-}$).

Figure 1B. Relationship between the reduction (Δ, calculated as the hypoxic minus the normoxic control value) in mitochondrial $PO_2$ ($\bar{P}_{O_2}^{mit}$) and increase in the cerebral output of ascorbate radicals ($A^{\bullet-}$).


A
HOCH2
HOCH
O
O
O
OH
Ascorbate monoanion
(AH-)
R•
(E0' > 282 mV)
-e-/-H+
+e-/+H+
HOCH2
HOCH
O
O
O
O
Ascorbate radical
(A•-)
NORMOXIA
Arterial Venous
HYPOXIA
Arterial Venous
Signal amplitude (AU)
2000
1500
1000
500
0
-500
-1000
-1500
-2000
2G
2169
2246
2432
3844
B
ΔP̄ O2 mit (mmHg)
-35
-30
-25
-20
-15
-10
-5
0
-20000
-40000
-60000
-80000
ΔA•- (AU 100g-1 min-1)
r = 0.82
P < 0.05